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Research Article

In silico Studies on the Inhibitory Nature of GTF -231(Gymnemic Acid, Trigonelline and Ferulic Acid in the ratio of 2:3:1), an Ayurvedic Preparation on the Activity of Phosphoenol Pyruvate Carboxykinase

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ABSTRACT

The prevalence of diabetes and its secondary complications are increasing globally due to genetic and environmental factors. T2DM accounts for more than 90% of the total diabetic population and its incidence is linked to obesity induced insulin resistance followed by insufficient secretion of insulin. Most of the currently available drugs for the treatment of T2DM are known to elicit undesirable side effects in addition to the development of resistance after prolonged use. Hence, the search for lead molecules preferably from plant origin continues. Recently, we have formulated a herbal preparation comprising of three phytochemicals namely Gymnemic acid (GA), Trigonelline (TG) and Ferulic acid (FA) in the ratio of 2:3:1 and studied its antidiabetic efficacy in type 2 diabetes in rats. Gluconeogenesis plays a pivotal role in the regulation of carbohydrate metabolism by synthesizing glucose from non-carbohydrate sources especially from the amino acids and its role is chiefly controlled by the activity of phosphoenolpyruvate carboxykinase (PEPCK). The present study is aimed to perform molecular docking studies involving the inhibitory effect of the above phytochemicals on PEPCK activity. Auto-Dock Tools were used to study the docking simulations. Auto Dock 4.2 is used to study the molecular interactions between the phytochemical ligands and the enzyme receptor, PEPCK. The binding energy obtained for GA, TG and FA on PEPCK is found to be –2.9, –5.92 and –7.2 Kcal/Mol, respectively. The data obtained suggested the inhibitory role of GTF-231 on the activity of PEPCK which in turn aids in the regulation of normoglycemia.

Keywords: Diabetes mellitus, Molecular docking, Phosphoenol pyruvate carboxykinase, Gymnemic acid, Trigonelline, Ferulic acid.

INTRODUCTION

Diabetes mellitus is a multifactorial, multisystemic and non-communicable metabolic disorder characterized by a chronic elevation in the levels of fasting and postprandial blood glucose. It is associated with impairment in the regulation of carbohydrate, protein and lipid metabolism which arises due to absolute lack of insulin secretion (T1DM) or insufficient insulin secretion coupled with insulin resistance (T2DM). More than 90% of the diabetic individuals belong to T2DM and its prevalence is increasing alarmingly at a rate of 3% per year. The contributory factors associated with chronic hyperglycemia in diabetes mellitus include impairment in glucose transport and its utilization due to insulin insufficiency and/or its resistance in peripheral tissues, excessive glucose production through gluconeogenesis in hepatic and non-hepatic tissues, decreased storage and increased degradation of glycogen and absorption of glucose and other nutrients in the small intestine. Drugs capable of maintaining normoglycemia through various pharmacological activities such as controlling the absorption of glucose in the intestine, decrease the insulin resistance, stimulation of insulin secretion, regulation of gluconeogenesis and glycogen metabolism are currently available for the treatment of T2DM. Several drugs are being therapeutically used either as monotherapy or in combinations to modulate the above biochemical processes to achieve normoglycemia in diabetic individuals. The synthesis of glucose from non-carbohydrate sources through the process of gluconeogenesis chiefly contribute to the chronic hyperglycemia in diabetes which in turn is an insulin dependent process.

Phosphoenolpyruvate carboxykinase (PEPCK-EC 4.1.1.32) is considered as an essentially critical enzyme in the regulation of gluconeogenesis because of its role in the hepatic glucose output and plays a vital role in the initiation, progression and development of diabetes and its secondary complications[1,2]. It is an established fact that the PEPCK gene expression in the liver is induced by fasting or by a diet devoid of carbohydrates due to a decrease in insulin levels and an increase in glucagon levels, the characteristic features of the fasted state[3]. The powerful negative effect of insulin on PEPCK- C gene transcription has been well established[4-6]. PEPCK exists in two isoforms in mammals namely, the mitochondrial (PEPCK-M) and the cytosolic form (PEPCK-C). PEPCK-M was originally identified from the chick liver mitochondria by Utter and Kurahashi[7]. PEPCK-C was described by Nordie and Lardy from the rats[8]. The genes for both isoforms are found to be

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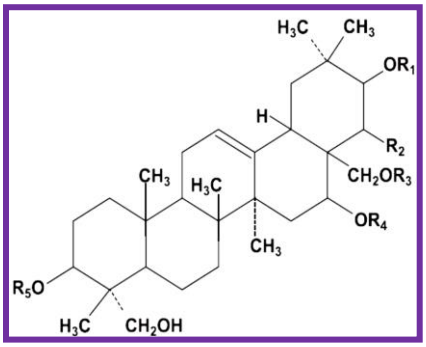
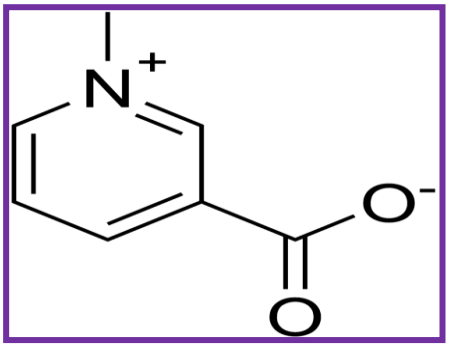
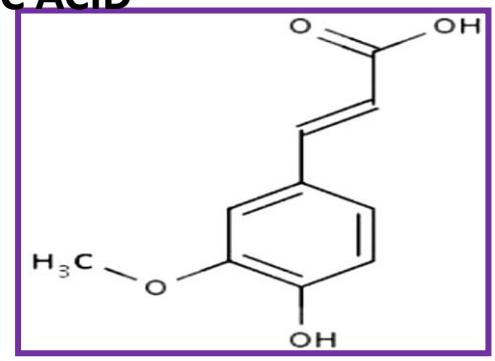


GYMNEMIC ACID

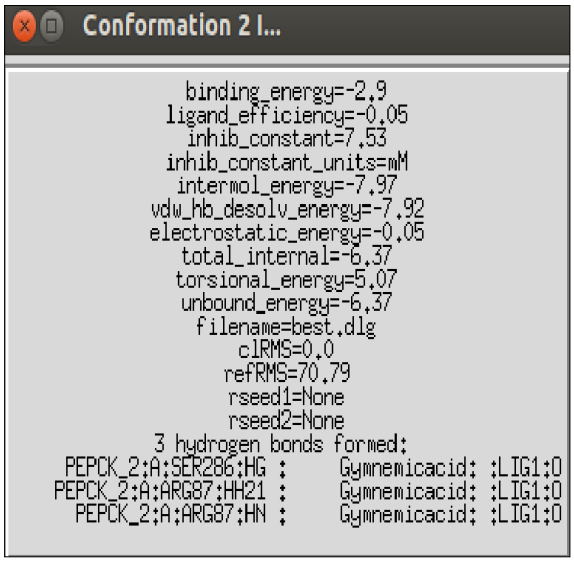


TRIGONELLINE



FERULIC ACID

Figure 1: Molecular structures of phytoligands.



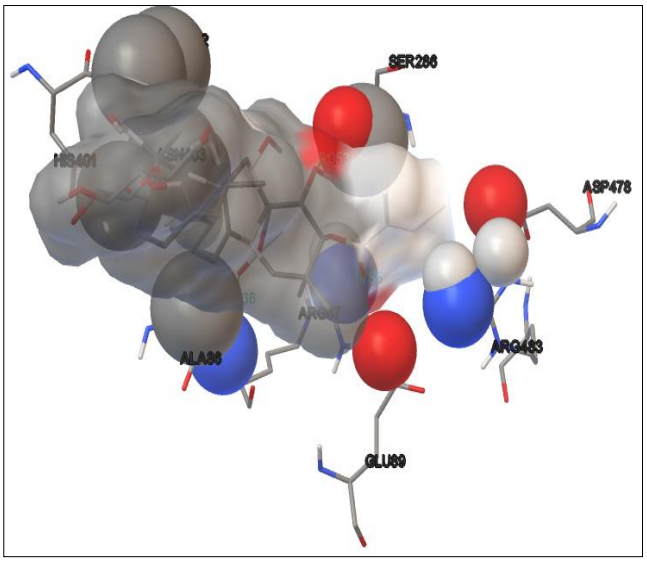


Figure 2: Docking conformation of Gymnemic acid with PEPCK using Auto Dock.

Table 1: Docking energy for gymnemic acid with PEPCK.

PEPCK		Gymnemic Acid	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
ARG89	N	O	2.83	–2.9
ARG89	NH2	O	2.58	

present in all eukaryotics. Equal distribution of the PEPCK isoforms has been reported in the liver tissues of human and most mammalian species[9]. Both the isoforms have the same molecular weight and catalyze similar reactions with similar kinetic properties, though they are coded for by different nuclear genes[10-12].

PEPCK catalyzes the following reaction:

OXALOACETATE + GTP ↔ PHOSPHOENOL PYRUVATE +CO_2+ GDP

The oxaloacetate required for the reaction is generated in the mitochondria from pyruvate by pyruvate carboxylase or synthesized by the cytosolic form of malate dehydrogenase as part of a shuttle that carries reducing equivalents (NADH) from the mitochondria to the

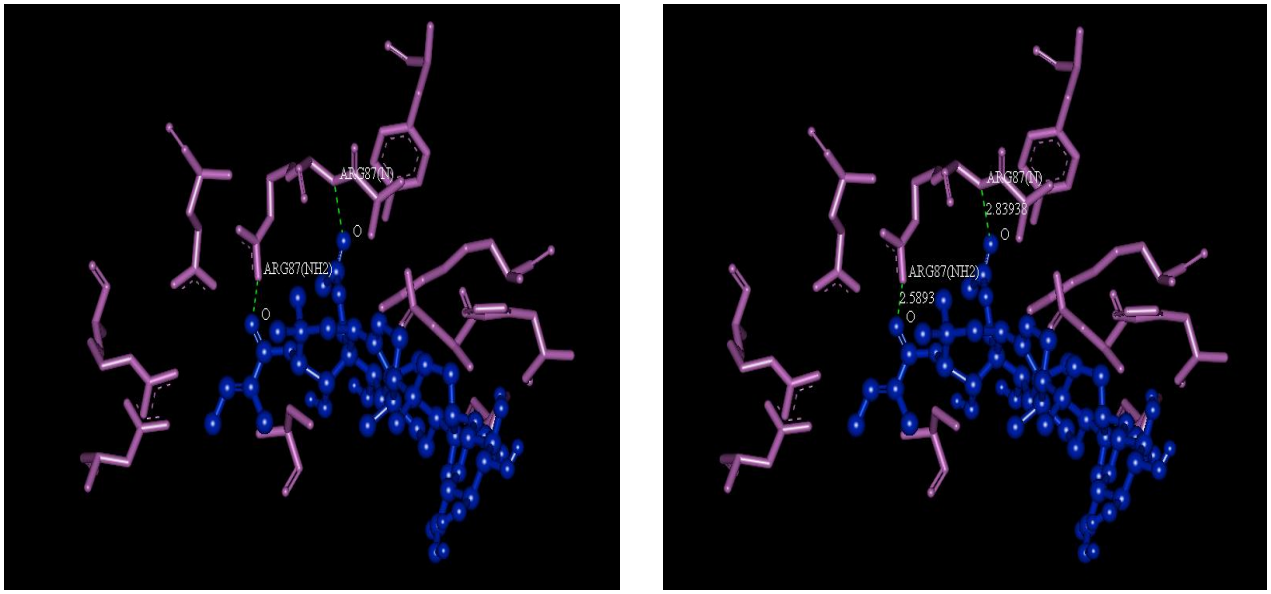


Figure 3: Hydrogen bonds interaction between PEPCK and Gymnemic acid using acceryls discovery studio visualizer.

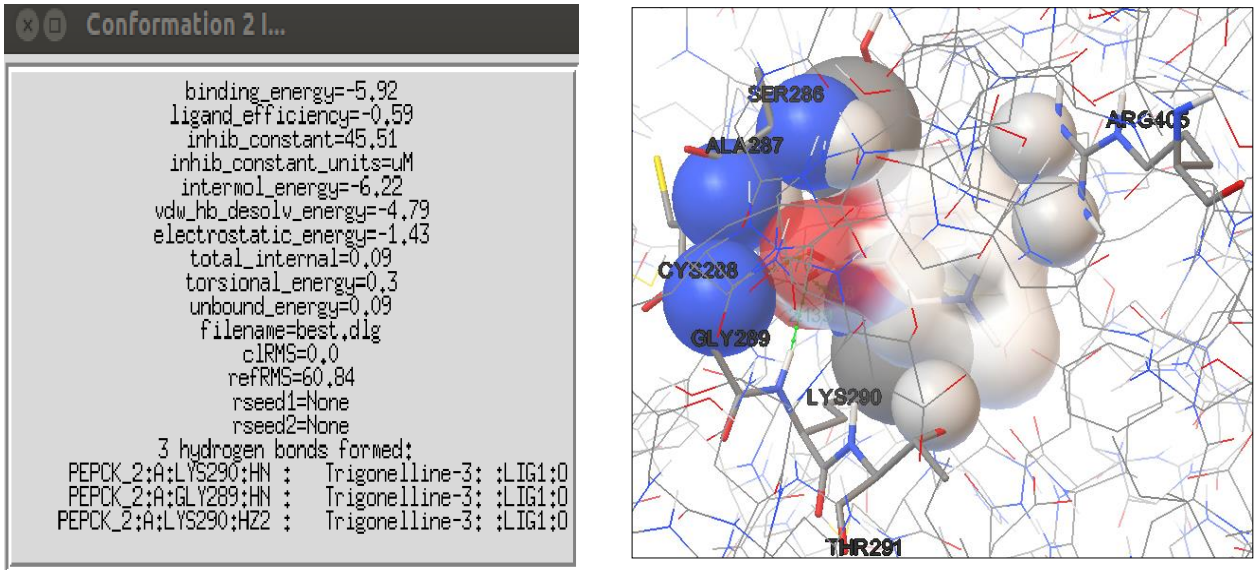


Figure 4: Docking conformation of Trigonelline with PEPCK using Auto Dock.

Table 2: Docking energy for Trigonelline with PEPCK.

PEPCK		Trigonelline	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
LYS290	NZ	O	2.97	–5.92
LYS290	N	O	3.15	
CYS288	N	O	3.03	
CYS288	N	O	3.10	
GLY289	N	O	2.77	
GLY289	N	O	2.95	

cytosol[13]. PEPCK-C appears in the liver tissue of mammals at birth indicating its role in glucose homeostasis in the prenatal period and it has a relatively short half life of about 6 hours[14] whereas PEPCK-M is present in the liver before birth[15] and its expression is initiated by the increase in hepatic cAMP levels and the fall in circulating insulin that occurs at birth[16]. It has been reported that the PEPCK-C gene expression can be induced to 10 fold in 30 minutes by cAMP administration to carbohydrate fed rats[17], whereas insulin administration causes a 50% reduction in transcription over the same time period[18]. The other regulators of PEPCK-C gene transcription include thyroid hormone, retinoic acid, epinephrine, high glucose levels and metabolic acidosis[19-21].

Transgenic animals that are over expressed for PEPCK-C contain significantly increased the levels of PEPCK-C mRNA in their liver and consequently a greatly elevated level of hepatic glucose output and a concomitant increase

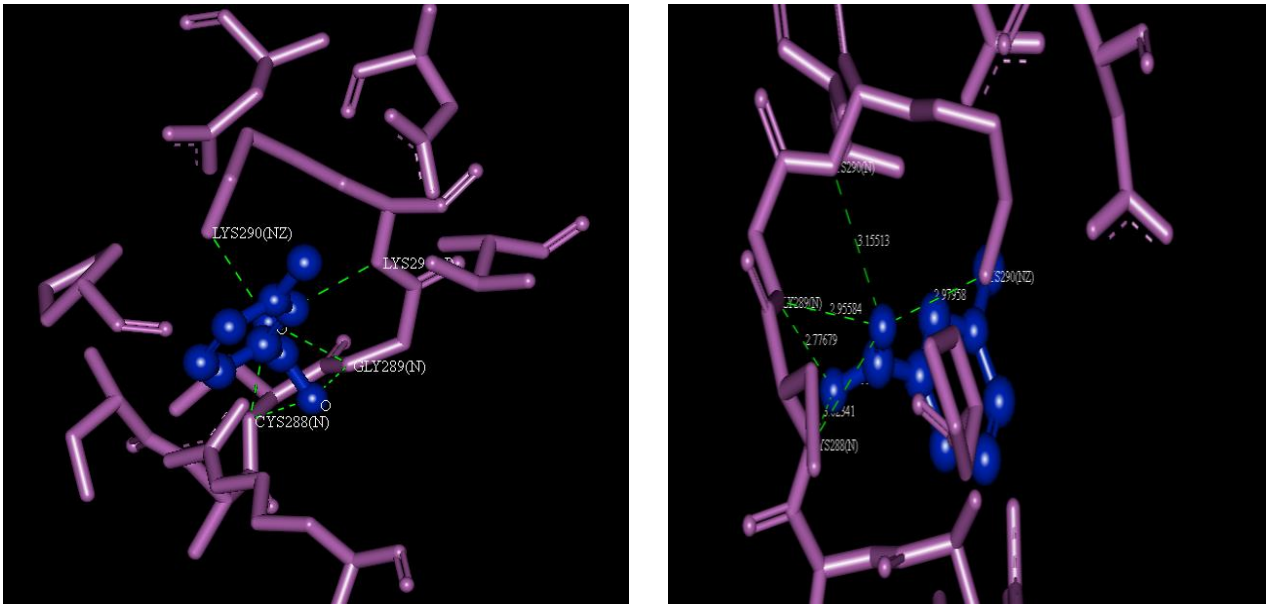


Figure 5: Hydrogen bonds interaction between PEPCK and Trigonelline using acceryls discovery studio visualize.

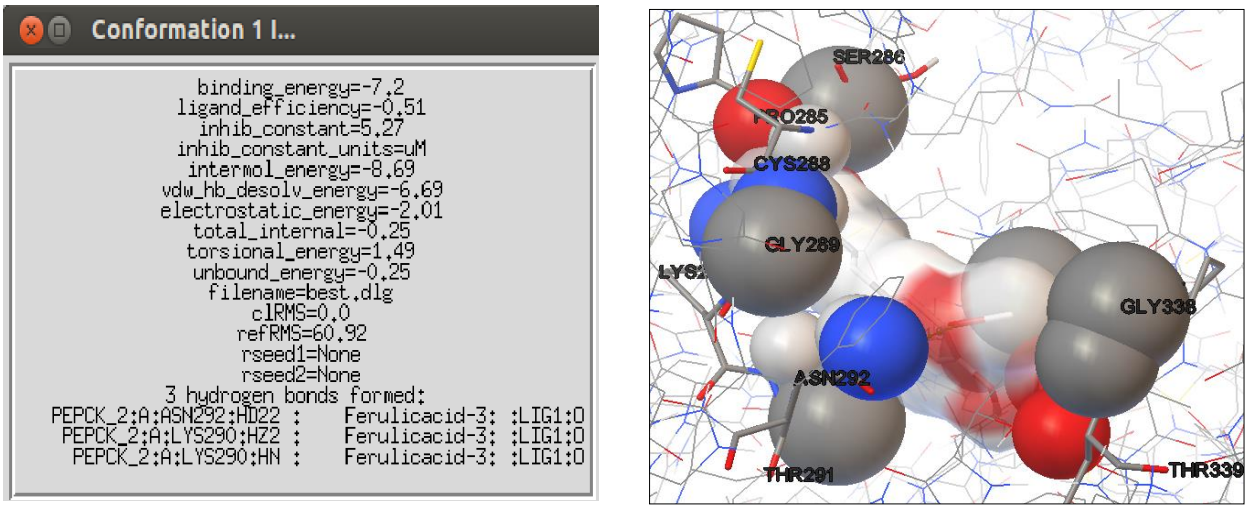


Figure 6: Docking conformation of Ferulic acid with PEPCK using Auto Dock.

in the level of blood glucose[2]. Similarly, the transgenic animals that are deleted for PEPCK-C gene exhibited severe hypoglycemia and mortality within 2 days after birth due to profound fatty livers[22]. These findings are considered as clinically important because the control of hepatic glucose output is a major therapeutic goal in achieving normoglycemia in diabetic individuals. Because DM is a chronic metabolic disorder that involves the impairment of both carbohydrate and lipid metabolism, it is essential to view PEPCK-C as a target for antidiabetic drugs.

MATERIALS AND METHODS

Preparation of ligands

The phytochemicals used in the present study namely Gymnemic acid, Trigonelline and Ferulic acid were considered as ligand molecules. The phytochemicals were constructed using Chemsketch and subsequently converted into the protein database (PDB) file format by adding the hydrogen bonds.

Preparation of receptor protein

Crystal structure of PEPCK was retrieved from RCSB PDB. Preparation of PEPCK with the Auto Dock Tools involves the addition of hydrogen atoms to the target enzyme for the preparation of protein docking simulation.

Molecular docking using Auto Dock

Auto-Dock Tools were used to study the docking simulations[23]. Auto Dock 4.2 is used to study the molecular interactions between the phytochemical ligands such as Gymnemic acid, Trigonelline and Ferulic acid and the enzyme receptor, PEPCK. Auto Dock requires pre-calculated grid maps, one for each type of atom present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 126, 126 and 126 Å (x, y and z) to include all the amino acid residues which are present in rigid macromolecules. Auto Grid 4.2 Program, supplied with Auto Dock 4.2 was used to produce grid maps. The spacing

Table 3: Docking energy for Ferulic acid with PEPCK.

PEPCK		Ferulic Acid	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
LYS290	NZ	O	2.66	–7.2
LYS290	NZ	O	2.96	
LYS290	N	O	3.16	
PRO285	O	H	1.97	
THR339	OG1	O	2.56	
THR339	OG1	H	2.42	
VAL335	O	H	2.26	
ASN292	ND2	O	2.72	
CYS288	N	O	3.12	
GLY289	N	O	2.98	

between grid points was 0.375 angstroms.

Auto Dock offers a variety of search algorithms to explore a given docking problem. In the present study, the Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The population size was set to 150 and the individuals were initialized randomly. A maximum number of energy evaluations were set to 500000, the maximum number of generations 1000, the maximum number of the top individual that automatically survived set to 1, the mutation rate of 0.02, the crossover rate of 0.8, Step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 0.5Aº, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed.

Auto Dock results were critically analyzed to study the interactions and the binding energy of the docked structure. It was run several times to obtain various docked conformations and to analyze the predicted docking energy. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were visualized using the Acceryls Visualizer discovery studio tool.

RESULTS AND DISCUSSION

It was recognized from the time of its discovery in the late 1950s that PEPCK was a key enzyme in the process of gluconeogenesis[1] and its activity is inhibited by the hormone insulin via a rapid and profound transcription regulation[24-29]. Yang et al., reported that the cytosolic PEPCK gene transcription is inhibited by phytochemicals such as resveratrol which activates SIR1, thereby deacetylating and inactivating HNF-4α, a key transcription factor involved in regulation of gene transcription[30]. Since PEPCK-C is a major regulator of gluconeogenesis, the effects of resveratrol and its analogs on transcription of the PEPCK gene expression are part of a drug development program aimed to achieve normoglycemia in T2DM[31]. Recent structural studies on the mitochondrial as well as cytosolic isozymes of PEPCK, evidenced the importance of conformational changes in the mechanism of PEPCK catalysis[32-34]. It is our hope that the *in silico* findings on the inhibitory role of PEPCK by the phytochemicals namely gymnemic acid, trigonelline and ferulic acid may pave a role in the designing of new agents for maintaining normoglycemia in T2DM patients.

The molecular structures of the phytochemicals as ligands were depicted as Figure 1. Figure 2 illustrates the docking conformation of gymnemic acid with PEPCK. The interactions of hydrogen bonding between PEPCK and gymnemic acid were visualized as Figure 3. Table 1 represents the data on the docking energy and the bonding distance between gymnemic acid and PEPCK.

The stability of Gymnemic acid with PEPCK is achieved by the hydrogen bonds of bond length 2.83 and 2.58Å with the residues ARG89 and ARG89 of PEPCK, respectively. The binding energy for Gymnemic acid with PEPCK was computationally calculated and found to be –2.9 Kcal/mol. Figure 4 exemplifies the docking conformation of trigonelline with PEPCK. The interactions of hydrogen bonding between PEPCK and trigonelline were visualized as Figure 5. Table 2 represents the docking energy and the bonding distance between trigonelline and PEPCK.

The trigonelline with PEPCK is stabilized by the hydrogen bonds of bond length 2.97, 3.15, 3.03, 3.10, 2.77 and 2.95Å with the residues LYS290, LYS290, CYS288, CYS288, GLY289 and GLY289 of PEPCK, respectively. The binding energy for trigonelline with PEPCK was derived computationally and found to be –5.92 Kcal/mol.

Figure 6 depicts the docking conformation of ferulic acid with PEPCK. The interactions of hydrogen bonding between PEPCK and ferulic acid were visualized as Figure 7. Table 3 represents the docking energy and the bonding distance between ferulic acid and PEPCK.

The ferulic acid with PEPCK is stabilized by the hydrogen bonds of bond length 2.66, 2.96, 3.16, 1.97, 2.56, 2.42, 2.26, 2.72, 3.12 and 2.98Å with the residues LYS290, LYS290, LYS290, PRO285, THR339, THR339, VAL335, ASN292, CYS288 and GLY289 of PEPCK, respectively. The binding energy for ferulic acid with PEPCK was calculated computationally and found to be –7.2 Kcal/mol.

Since the discovery of PEPCK in 1953, the studies on the biological actions of this enzyme have increased exponentially. The role of PEPCK has been linked virtually exclusively to gluconeogenesis. The significant negative effect of insulin on PEPCK gene expression has been well documented. Earlier, we have reported the antidiabetic and other pharmacological properties of gymnemic acid trigonelline and ferulic acid in high fat diet fed low dose STZ induced type 2 diabetes in rats[35,36]. The

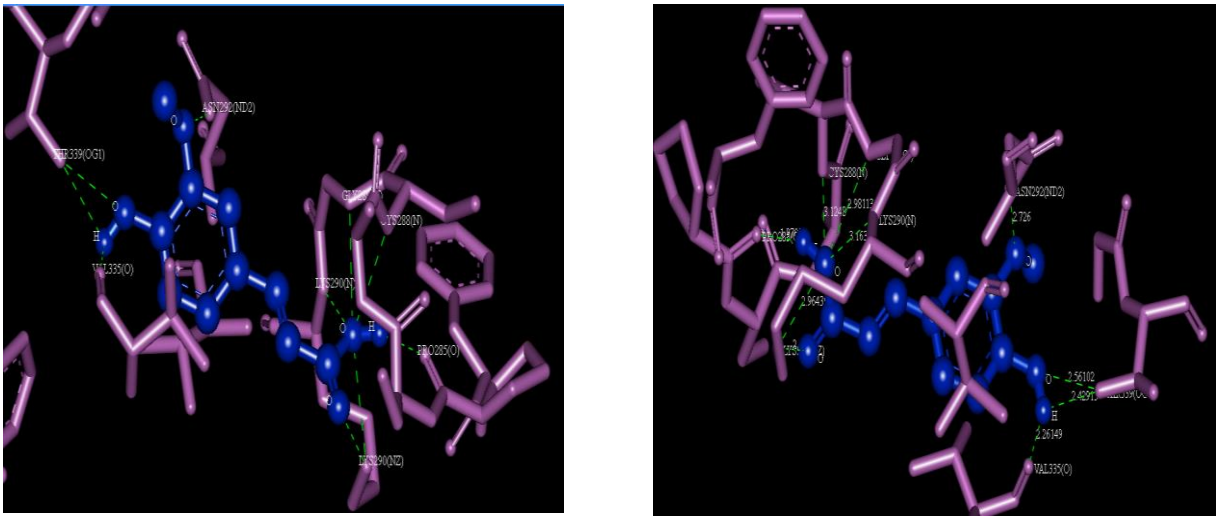


Figure 7: Hydrogen bonds interaction between PEPCK and Ferulic acid using acceryls discovery studio visualize.

results of the present study provide a substantial evidence for the PEPCK inhibitory effect of the above phytochemicals which in turn may be responsible for their antidiabetic properties.

CONCLUSION

The data obtained through the molecular docking studies of the phytoligands such as gymnemic acid, trigonelline and ferulic acid with phosphoenol pyruvate carboxykinase evidenced the exceptional inhibitory potential of the phytochemicals. The above plant derived secondary metabolites are reported to have significant pharmacological properties in addition to their non-toxic nature. Since all the three phytochemicals have shown highest binding energy and affinity to bind the molecular markers, the present study may pave a new pathway in the development of active pharmacophore discovery and offering novel insights into the therapeutics to regulate gluconeogenesis in diabetic individuals to maintain normoglycemia.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Kurahashi K, Semenza G. Comprehensive Biochemistry: Selected Topics in the History of Biochemistry: Personal Recollections, Elsevier Science Co. 1986, 36, 71–97.
2. Valera A, Pujol A, Pelegrin M, Bosch F. Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci 1994; 91: 9151–9154.
3. Reshef L, Hanson RW. The interaction of catecholamines and adrenal corticosteroids in the induction of phosphopyruvate carboxylase in rat liver and adipose tissue. Biochem J 1972; 127: 809–818.
4. Zorzoli A, Turkenkopf IJ, Mueller VL. Gluconeogenesis in developing rat kidney cortex. Biochem J 1969; 111: 181–185.
5. Patel MS, van Lelyveld P, Hanson RW, Jones CT. The Biochemical Development of the Fetus and the Neonate, Elsevier Science Publishing Co. 1982, 553–571.
6. Granner DK, Andreone T, Sasaki K, Beale E. Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. Nature 1983; 305: 549–551.
7. Utter MF, Kurahashi K. Mechanism of action of oxaloacetate carboxylase from liver. J Am Chem Soc 1953; 7: 785–787.
8. Nordlie RC, Lardy HA. Mammalian liver phosphoneolpyruvate carboxykinase activities. J Biol Chem 1963; 238: 2259–2263.
9. Hanson RW, Garber AJ. Phosphoenolpyruvate carboxykinase I. Its role in gluconeogenesis. Am J Clin Nutr 1972; 25: 1010–1021.
10. Jomain-Baum M, Schramm VL. Kinetic mechanism of phosphoenolpyruvate carboxykinase (GTP) from rat liver cytosol. Product inhibition, isotope exchange at equilibrium, and partial reactions. J Biol Chem 1978; 253: 3648–3659.
11. Lee MH, Hebda CA, Nowak T. The role of cations in avian liver phosphoenolpyruvate carboxykinase catalysis. Activation and regulation. J Biol Chem 1981; 256: 12793–12801.
12. Hebda CA, Nowak T. Phosphoenolpyruvate carboxykinase. Mn2+ and Mn2+ substrate complexes. J Biol Chem 1982; 257: 5515–5522.
13. Hanson RW, Patel YM, Meister A, Ed. Advances in Enzymology, Vol. 69, John Wiley and Sons, New York, pp. 1994; 203–281.
14. Ballard FJ, Hanson RW. Phosphoenolpyruvate carboxykinase and pyruvate carboxylase in developing rat liver. Biochem J 1967; 104: 866–871.

15. Arinze IJ. On the development of phosphoenolpyruvate carboxykinase and gluconeogenesis in guinea pig liver. Biochem Biophys Res Commun 1975; 65: 184–189.
16. Garcia-Ruiz JP, Ingram R, Hanson RW. Changes in hepatic messenger RNA for phosphoenolpyruvate carboxykinase (GTP) during development. Proc Natl Acad Sci 1978; 75: 4189–4193.
17. Lamers WH, Hanson RW, Meisner HM. cAMP stimulates transcription of the gene for cytosolic phosphoenolpyruvate carboxykinase in rat liver nuclei. Proc Natl Acad Sci 1982; 79: 5137–5141.
18. Granner DK, Andreone T, Sasaki K, Beale E. Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. Nature 1983; 305: 549–551.
19. Meyuhas O, Reshef L, Ballard FJ, Hanson RW. The effect of insulin and glucocorticoids on the synthesis and degradation of phosphoenolpyruvate carboxykinase (GTP) in rat adipose tissue cultured *in vitro*. Biochem J 1976; 158: 9–16.
20. Iynedjian PB, Hanson RW. Messenger RNA for renal phosphoenolpyruvate carboxykinase (GTP). Its translation in a heterologous cell-free system and its regulation by glucocorticoids and by changes in acid-base balance. J Biol Chem 1977; 252: 8398–8403.
21. Hanson RW, Reshef L. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. Annu Rev Biochem 1997; 66: 581–611.
22. She P, Shiota M, Shelton KD, Chalkley R, Postic C. Phosphoenolpyruvate carboxykinase is necessary for the integration of hepatic energy metabolism. Mol Cell Biol 2000; 20: 6508–6517.
23. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010; 31: 455-461.
24. Sasaki K, Cripe TP, Koch SR, Andreone TL, Petersen DD, Beale EG, Granner DK. Multihormonal regulation of phosphoenolpyruvate carboxykinase gene transcription. The dominant role of insulin. J Biol Chem 1984; 259:15242–51.
25. Magnuson MA, Quinn PG, Granner DK. Multihormonal regulation of phosphoenolpyruvate carboxykinase-chloramphenicol acetyltransferase fusion genes. Insulin's effects oppose those of cAMP and dexamethasone. J Biol Chem 1987; 262(31):14917-20.
26. Pilkis SJ, el-Maghrabi MR, Claus TH. Hormonal regulation of hepatic gluconeogenesis and glycolysis.Annu Rev Biochem 1988; 57: 755–83.
27. O' Brien RM and Granner DK. Regulation of gene expression by insulin. Biochem J 1991; 278: 609–19.
28. Pilkis SJ, Granner DK. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 1992; 54: 885–909.
29. O' Brien RM, Streeper RS, Ayala JE, Stadeknaier BT, Hombuckle LA. Insulin-regulated gene expression. Biochem Soc Trans 2001; 29: 552-558.
30. Yang J, Kong X, Martins-Santos ME, Aleman G, Chaco E, Liu GE, Wu SY, Samols D, Hakaimi P, Chiang CM, Hanson RW. Activation of SIRT1 by resveratrol represses transcription of the gene for the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) by deacetylating hepatic nuclear factor 4alpha. J Biol Chem 2009; 284: 27042–27053.
31. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 2007; 450L: 712–716.
32. Holyoak T, Sullivan SM, Nowak T. Structural insights into the mechanism of PEPCK catalysis. Biochemistry 2006; 45: 8254–8263.
33. Sullivan SM, Holyoak T. Structures of rat cytosolic PEPCK: insight into the mechanism of phosphorylation and decarboxylation of oxaloacetic acid. Biochemistry 2007; 10078–10088
34. Sullivan M, Holyoak T. Enzymes with lid-gated active sites must operate by an induced fit mechanism instead of conformational selection. Proc Natl Acad Sci 2008; 105: 13829–13834
35. Roshana Devi V, Sriram Prasath G, Subramanian S. Antidiabetic properties of GTF – 231, an ayurvedic formulation studied in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats. Der Pharmacia Lettre 2015; 7: 113-123.
36. Roshana Devi V, Subramanian S. Biochemical Evaluation of Hypoglycemic Effect of GTF-231, a Polyherbal Preparation in High Fat Diet Fed- Low Dose STZ Induced Experimental Type 2 Diabetes in Rats. Der Pharmacia Lettre 2016; 8: 121-132.